Elevated Serum Glucose-6-Phosphate Isomerase Correlates with Histological Disease Activity and Clinical Improvement After Initiation of Therapy in Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To determine serum glucose-6-phosphate isomerase (GPI) concentrations in patients with rheumatoid arthritis (RA), and to test whether they correlate with objective measures of disease activity.

Methods. Sera from 116 patients with RA, 69 patients with non-RA rheumatic diseases, and 101 healthy controls were analyzed. Levels of soluble serum GPI were measured by ELISA. Histological disease activity was determined with the synovitis score in synovial needle biopsies from 58 of the 116 patients with RA. Thirty-one of the 58 synovium samples were stained for CD68, CD3, CD20, CD38, CD79a, and CD34 by immunohistochemistry. Demographic data were collected, as well as serological and clinical variables that indicate RA disease activity, for Spearman correlation analysis.

Results. Serum GPI level correlated positively with the synovitis score ($r = 0.278$, $p = 0.034$). Significantly higher soluble GPI levels were detected in the RA sera compared with sera from healthy controls and the non-RA disease controls (2.25 ± 2.82 vs 0.03 ± 0.05 and 0.19 ± 0.57 μg/ml, respectively; $p < 0.0001$). The rate of serum GPI positivity was significantly higher in the RA patients than in the non-RA disease controls (64.7% vs 10.1%; $p < 0.0001$). Spearman analysis showed no significant correlation between serum GPI level and Disease Activity Score in 28 joints at baseline. After initiation of antirheumatic treatments, GPI levels decreased significantly (2.81 ± 3.12 vs 1.44 ± 2.09 μg/ml; $p = 0.016$), paralleling improvement of the disease activity indices.

Conclusion. Elevated serum GPI may be involved in the synovitis of RA and may prove useful as a serum marker for disease activity of RA. (First Release September 1 2010; J Rheumatol 2010;12:2452–61; doi:10.3899/jrheum.100157)

Key Indexing Terms:
GLUCOSE-6-PHOSPHATE ISOMERASE
DAS28 SCORE
RHEUMATOID ARTHRITIS
SYNOVITIS

Rheumatoid arthritis (RA) is an autoimmune disease that manifests as chronic inflammation of the joints. The pathogenesis of RA is multifactorial, including genetic influences on susceptibility, environmental factors, immune mechanisms, and amplifying cytokine networks that perpetuate inflammation[1,2,3,4]. This process is characterized by an increased presence of monocytes, macrophages, and lymphocytes in the synovial fluid (SF) and tissue, leading to release of many cytokines and chemokines. These proinflammatory mediators subsequently activate proteases, culminating in the destruction of bone and cartilage, which leads to increased disability in patients with RA[5]. The precise mechanism of disease development remains unclear. Although the etiology of RA is presumed to be an autoimmune reactivity to antigens that are specifically expressed in joints, an alternative possibility is that an ubiquitously expressed antigen could be modified and exposed in the joints as a neoepitope[6].

Glucose-6-phosphate isomerase (GPI), also known as phosphoglucose isomerase, is one of the well known glycolytic enzymes that catalyzes the interconversion of glucose-6-phosphate and fructose-6-phosphate. In addition to the enzymatic function, it is well established that mammalian GPI can act as extracellular cytokines such as autocrine motility factor (AMF)[7], neurokine[8,9], and matura-

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Supported by Chinese National Natural Science Research Grant (no. 30972742) to Prof. Dai.

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Accepted for publication July 14, 2010.

tion factor[10]. Matsumoto, *et al* have proposed GPI as a novel autoantigen in RA[11]. K/BxN T cell receptor transgenic mice spontaneously develop a destructive and chronic polyarthritis that shares features with human RA. It has been proposed that GPI serves as an autoantigen for both B and T cells in this model, and adoptive transfer of anti-GPI antibodies from K/BxN mice to naive mice can induce inflammatory arthritis with features similar (but not identical) to those of human RA[11]. In addition, immunization of genetically unaltered mice with GPI has been shown to induce peripheral polyarthritis[12]. These mice produced autoantibodies directed against the ubiquitous cytoplasmic GPI, which induced arthritis when injected into normal recipients[13].

Schaller, *et al* detected anti-GPI IgG in a large proportion of patients with RA but rarely in patients with Sjögren's syndrome, Lyme arthritis, or osteoarthritis (OA), or in healthy subjects matched for age and sex[13]; and elevated levels of anti-GPI antibodies have been associated with more severe forms of RA[14]. The presence of anti-GPI antibodies in sera from RA patients has been confirmed by others, but recent data suggest a lack of diagnostic value of anti-GPI autoantibodies and their inability to predict radiological progression in early arthritis[15,16,17,18,19,20].

More recently it was shown that the systemic immune response against GPI could induce joint-specific pathology in genetically unaltered DBA/1 mice. More than 90% of these mice developed a severe symmetrical peripheral polyarthritis following a single immunization with recombinant human or murine GPI in adjuvant[12]. Schaller, *et al* had shown that the concentration of soluble GPI was elevated in sera and SF of RA patients, leading to immune complex formation[13]. They also demonstrated immunohistochemically that RA synovium expressed high concentrations of GPI on the surface of the synovial lining and the endothelium of arterioles[13]. Recently, these investigators found elevated serum levels of GPI enzymatic activity in a broad range of inflammatory arthritic diseases, while significantly higher concentrations of enzymatically inactive forms were present only in RA. Thus, they presumed that elevated GPI levels may contribute to elevated levels of anti-GPI antibodies and GPI/anti-GPI immune complexes, which in turn may trigger production of proinflammatory cytokines and perpetuate the inflammatory process[21].

However, the clinical significance of serum soluble GPI has not yet been assessed in a well documented cohort of RA patients. Our aim was to evaluate serum GPI levels in RA, and to determine whether these correlated with indices of RA disease activity.

MATERIALS AND METHODS

Patients. One hundred sixteen patients with RA who fulfilled the American College of Rheumatology 1987 criteria for RA[22] were recruited from the Department of Rheumatology of Sun Yat-Sen Memorial Hospital of Sun Yat-Sen University, Guangzhou. Twenty-four patients were newly diagnosed and were naive to treatment with prednisone or disease-modifying antirheumatic drugs (DMARD). Control blood samples were obtained from healthy donors (n = 101) who had no family history of RA, as well as 69 patients with other forms of non-RA rheumatic diseases [including gout (n = 15), ankylosing spondylitis (AS; n = 15), systemic lupus erythematosus (SLE; n = 11), OA (n = 7), unclassified connective tissue disease (n = 4), primary Sjögren's syndrome (pSS; n = 3), polymyalgia rheumatica (n = 3), undifferentiated spondyloarthritis (n = 4), dermatomyositis (n = 2), systemic sclerosis (n = 1), reactive arthritis (n = 1), Wegener's granulomatosis (n = 1), fasciitis (n = 1), and vasculitis (n = 1)] as defined according to established clinical criteria[23]. The demographic and drug treatment data of the subjects are shown in Table 1. All participants provided informed consent. The study was approved by the ethics committees and was performed in accord with the Declaration of Helsinki.

Synovitis assessment. Synovium from inflamed knees of 58 of the 116 RA patients was collected by closed-needle biopsy[24]. Samples were fixed in 10% neutral formalin, embedded in paraffin, cut in microsections of 4 μm, and stained with H&E according to routine procedures. The samples were evaluated by 2 pathologists according to a validated synovitis score[25,26,27,28,29]. Three features of chronic synovitis (hyperplasia of the lining cell layer, cellular density of the synovial stroma, inflammatory cell infiltration) were scored from 0 to 3, with the sum providing the synovitis score, which was interpreted as follows: 0–1, no synovitis; 2–4, low-grade synovitis; 5–9, high-grade synovitis. This score correlates positively with synovial proliferation and expression of the CD68 antigen, a well established synovial tissue biomarker for RA[29].

Immunohistochemistry. Thirty-one of the 58 synovium samples were studied by immunohistochemistry. Sections (5 μm thick) from paraffin blocks were stained for CD68 (macrophages; clone KP-1), CD3 (T cells; clone PS1), CD20 (B cells; clone L26), CD38 (plasma cells; clone SPC32), CD79a (B lineage cells from pre-B cell to plasmocyte stage; clone SP18), and CD34 (endothelial cells; clone QBEnd/10). Commercial antibody preparations (Invitrogen, Carlsbad, CA, USA) were used according to standard staining protocols. An automated immunostainer (PV-6000; Golden Bridge International, Los Angeles, CA, USA) was used with nonspecific isotype or IgG as negative control and diaminobenzidine as chromogen. Absence of staining due to technical failure was excluded by including appropriate positive control tissues in each staining run.

Quantitative analysis was performed to evaluate the densities of CD68+, CD3+, CD20+, CD38+, CD79a+, or CD34+ cells in these RA synovium samples. The numbers of CD68, CD3, CD20, CD38, CD79a, or CD34-expressing cells per high power field (400×) were determined in 5–12 fields per specimen by manual cell counting as described[30]. Only fields with clearly recognizable lining and subintimal vasculature were included. All results were converted to densities per mm^2 using the formula: positive staining cells/mm^2 = (positive staining cells/400× field) × 6.29.

Semiquantitative analysis was performed to evaluate the intensity of CD68+ cells in the lining layer of RA synovium as described[31]. CD68+ lining cells were scored on a scale of 0–4. A score of zero represented no or minimal infiltration, while a score of 4 represented intense infiltration.

Disease assessments. Disease assessments were performed at the time of recruitment into the study. Disease activity of RA was measured with the Disease Activity Score in 28 joints (DAS28)[31]. DAS28-CRP was calculated using the following formulas[32]: DAS28-CRP = (0.56 × sqrt(28TJC) + 0.28 sqrt(28SJC) + 0.36 × ln(CRP +1)) × 1.1 + 1.15, where TJC represents the tender joint count, SJC represents the swollen joint count, and CRP represents C-reactive protein. A DAS28-CRP between > 2.6 and < 3.2 is considered low disease activity, a value between > 3.2 and < 5.1 moderate activity, a value > 5.1 high activity, and a value < 2.6 remission. Patient self-assessed pain was scored on a visual analog scale (VAS; 0–100 mm)[33,34]. Disability status was reported by the patients using the Swedish version of the Stanford Health Assessment Questionnaire (HAQ)[35]. It consists of 20 items in 8 categories of activities of daily living. Total HAQ scores range from 0 to 3. Socioeconomic and psychosocial factors that contribute substantially to the patients' quality of life were assessed by the

Table 1. Demographic and clinical variables of the study subjects.

Variables	Healthy Controls, n = 101	Patients with RA, n = 116	Others, n = 69
Age, yrs			
Range	18–49	12–79	18–93
Mean (SD)	37.6 (9.2)	51.8 (14.2)	43.3 (20.4)
Gender, n (%) female	67 (66.3)	83 (71.6)	36 (52.2)
Disease duration, mean (SD), months	NA	79.4 (109.7)	61.0 (76.6)
DAS28-CRP, mean (SD)	NA	4.7 (1.4)	NA
Medications, n (%)			
Prednisone	0	73 (62.9)	21 (30.4)
Methotrexate	0	40 (34.5)	4 (5.8)
Leflunomide	0	20 (17.2)	2 (2.9)
Salicylazosulfapyridine	0	11 (9.5)	5 (7.2)
Hydroxychloroquine	0	5 (4.3)	3 (4.3)
Other DMARD	0	2 (1.7)	5 (7.2)
Etanercept	0	4 (3.4)	0
Infliximab	0	3 (2.6)	0

NA: not applicable; RA: rheumatoid arthritis; DAS28: Disease Activity Score in 28 joints; CRP: C-reactive protein; DMARD: disease modifying antirheumatic drugs; Others; other non-RA rheumatic diseases including gout (n = 15), ankylosing spondylitis (n = 15), systemic lupus erythematosus (n = 11), osteoarthritis (n = 7), unclassified connective tissue disease (n = 4), primary Sjögren's syndrome (n = 3), polymyalgia rheumatica (n = 3), spondyloarthritis (n = 4), dermatomyositis (n = 2), systemic sclerosis (n = 1), reactive arthritis (n = 1), Wegener's granulomatosis (n = 1), fasciitis (n = 1), and vasculitis (n = 1).

Arthritis Impact Measurement Scale (AIMS)[36,37]. Data on several clinical and biological measures were also collected: TJC of 28 joints, SJC of 28 joints, early morning stiffness, gripping power, erythrocyte sedimentation rate (ESR), CRP, and autoantibodies including rheumatoid factor (RF) and anticyclic citrullinated peptide (CCP) antibodies.

ELISA for determination of GPI concentration. Serum samples were collected from the patients and healthy controls after overnight fasting and stored at –80°C until analysis. Serum levels of soluble GPI were measured with a human GPI detection kit (Shanghai Beijia Biochemical Sciences Co., Ltd. Shanghai, China) according to the manufacturer's instructions. This detects soluble GPI in the form of GPI-antigen or GPI-anti-GPI complexes in the serum. Measurements were done in duplicates. Briefly, the serum samples were placed in anti-human GPI antibody-coated microtiter wells. In addition, serial dilutions of recombinant soluble human GPI (0.00–4.00 μg/ml) were added to the plate to construct a standard curve. The plates were then incubated 1 h at 37°C and washed with wash buffer 5 times. Then 100 μl of avidin-horseradish peroxidase (HRP) labeled murine anti-GPI antibody was added to each well and incubated 1 h at 37°C. The plates were washed, followed by addition of 100 μl developer solution per well, and incubated 15 min at room temperature. Then 50 μl of stop solution were added to each well. The optical density of each well was determined within 30 min, using a microplate reader set to 492 nm, with wavelength correction set to 630 nm. Soluble GPI concentrations < 0.2 μg/ml were considered to be negative. The assays were performed blindly, without knowledge of the patient's disease status or activity.

Statistical analysis. The statistical analysis was performed using SPSS for Windows 13.0 (SPSS Inc., Chicago, IL, USA). Data are presented as frequencies and percentages for categorical variables and mean ± SD for continuous variables, unless otherwise indicated. Because serum GPI levels were not distributed normally, nonparametric testing using the Mann-Whitney rank-sum test between 2 groups, or Kruskal-Wallis one-way analysis of variance on ranks among 3 or more groups for continuous variables, and the chi-square test for proportions was performed. For paired samples, the Wilcoxon signed-rank test was used. For assessing the correlation between serum GPI level and the TJC in 28 joints, SJC in 28 joints, ESR, CRP, RF, morning stiffness, gripping power, and DAS28-CRP, Spearman's rank order correlation test was used. All significance tests were 2-tailed and were conducted at the 5% significance level.

RESULTS

Disease activity in the RA patient population. Clinical and demographic characteristics of the 116 RA patients are shown in Table 1. Mean disease duration was 79.4 months (range 1–360). The mean DAS28-CRP score was 4.73 (range 1.65–7.57); 48 patients were in the high activity group, 50 the moderate, 9 in the low activity group, and 9 were in disease remission. Serological and clinical measures that reflect disease activity in these patients are shown in Table 2.

Pathological synovitis score and serum GPI level. In the 58 RA patients in whom the synovitis score had been assessed by H&E staining, the mean score was 3.5 (range 0.5~7.0). Only one patient had no synovitis (score, < 1), 45 had low-grade synovitis, and 12 had high-grade synovitis. Forty-four of the patients were female and 14 were male. The mean age was 50.7 years (range 12–78). The serum GPI level in these 58 RA patients was 2.36 ± 2.78 μg/ml. The serum GPI level in the low-grade synovitis and high-grade synovitis groups was 2.21 ± 2.82 μg/ml and 3.12 ± 2.69 μg/ml, respectively, but this difference was not significant ($p = 0.131$). In contrast, a significant correlation was found between serum GPI levels and the synovitis scores from all 58 RA patients ($r = 0.278$, $p = 0.034$; Figure 1A). When the 3 components of the score (hyperplasia of synovial lining, density of synovial stroma, and inflammatory cell infiltration) were studied separately, inflammatory cell infiltration was found to correlate best with serum GPI levels ($r = 0.358$, $p = 0.006$; Figure 1B, 1C, 1D).

Table 2. Serological and clinical measurements of the patients with RA.

Characteristic	Mean ± SD (range)
CRP, mg/l	34.8 ± 36.0 (0.17–193.0)
ESR, mm/h	64.0 ± 38.8 (4.0–145.0)
RF, U/ml	292.4 ± 477.4 (9.1–3010.0)
Anti-CCP, U/ml	132.1 ± 123.6 (1.4–300.0)
Swollen joint count of 28 joints	6.8 ± 6.6 (0.0–28.0)
Tender joint count of 28 joints	9.9 ± 8.4 (0.0–28.0)
DAS28-CRP	4.73 ± 1.41 (1.65–7.57)
Pain VAS, mm	5.2 ± 2.2 (0.0–10.0)
HAQ score	0.97 ± 0.70 (0.0–2.55)
AIMS	4.7 ± 2.5 (0.0–10.0)
Early morning stiffness, min	62.8 ± 87.8 (0.0–360.0)
Gripping power, mm Hg	74.1 ± 31.5 (20.0–230.0)
Sera GPI, μg/ml	2.25 ± 2.82 (0.01–11.32)

RA: rheumatoid arthritis; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; RF: rheumatoid factor; Anti-CCP: anticyclic citrullinated peptide antibodies; DAS28: Disease Activity Score in 28 joints; HAQ: Health Assessment Questionnaire; AIMS: Arthritis Impact Measurement Scales; GPI: glucose-6-phosphate isomerase; VAS: visual analog scale.

Evaluation of H&E-stained sections showed that there were more mononuclear inflammatory cells in the synovium of patients with higher serum GPI levels compared with patients with low serum GPI levels (Figure 2A, 2B). Similarly, patients with higher serum GPI level had more CD68+ cells in the synovium (Figure 2C, 2D). A modest positive correlation was detected between the serum GPI level and CD68+ staining in the lining layer of RA synovium ($r = 0.364$, $p = 0.048$). No significant correlation was found between serum GPI level and CD3+, CD20+, CD38+, CD79a+, or CD34+ cell density (all $p > 0.05$). However, the density of the common mononuclear inflammatory cell types, CD68+ cells, CD3+ cells, CD38+ cells, and CD20+ cells, correlated positively with the synovitis score ($r = 0.504$, $r = 0.683$, $r = 0.787$, and $r = 0.805$, respectively; $p = 0.009$, $p < 0.001$, $p < 0.001$, and $p < 0.001$, respectively). CD34+ and CD79a+ cell densities also correlated positively with the synovitis score ($r = 0.520$ and $r = 0.779$; $p = 0.005$ and $p < 0.001$).

GPI levels in sera. To determine whether serum level of GPI was altered in RA patients in general, we determined GPI levels in a blinded manner in sera from additional RA patients, as well as in various non-RA rheumatic disease controls and healthy controls. Significantly higher soluble GPI was detected in serum samples of RA patients compared to healthy controls: RA patients, 2.25 ± 2.82 μg/ml; healthy

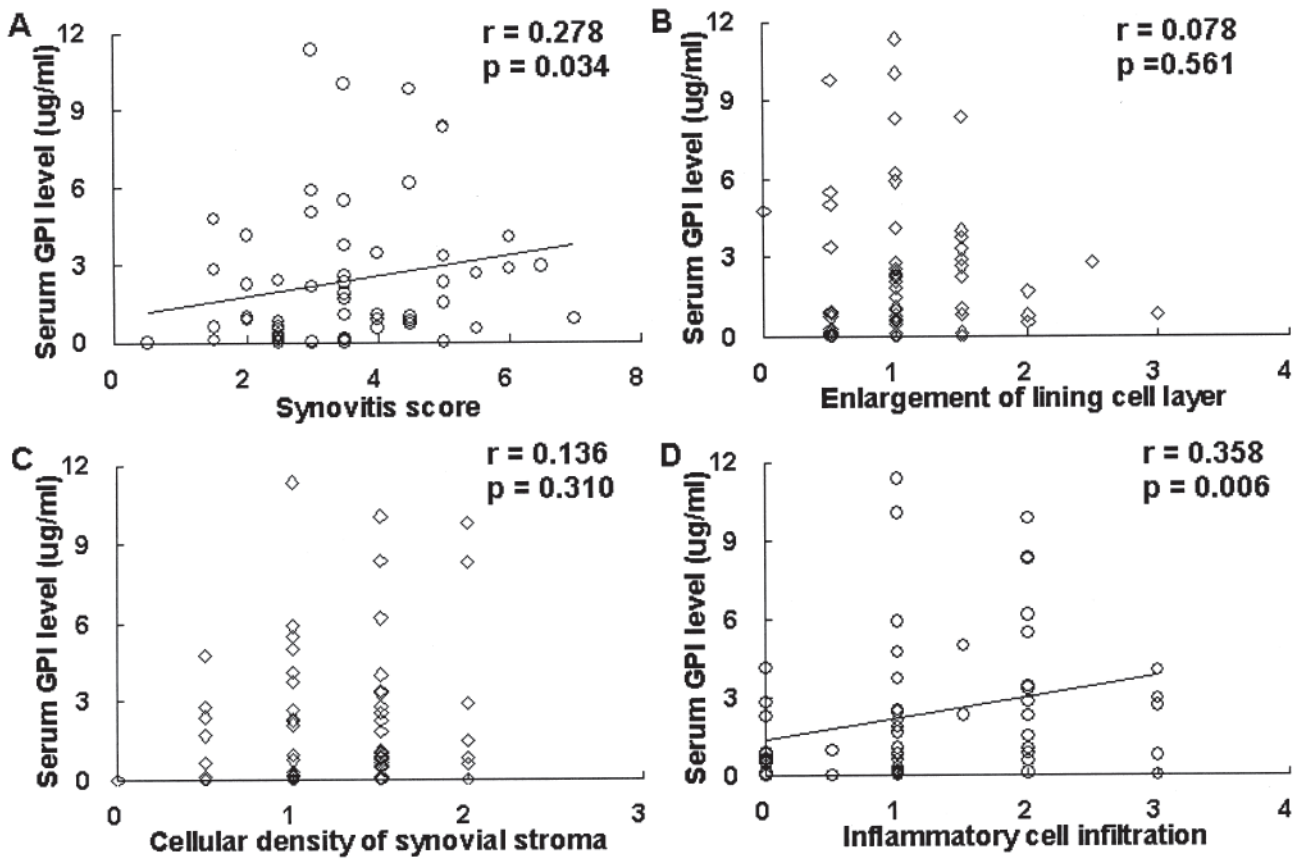

Figure 1. Spearman rank correlation analysis for serum GPI level and synovitis score in RA. A. Correlation between serum GPI level and synovitis score. B. Correlation between serum GPI level and hyperplasia of the lining cell layer. C. Correlation between serum GPI level and cellular density of synovial stroma. D. Correlation between serum GPI level and inflammatory cell infiltration.

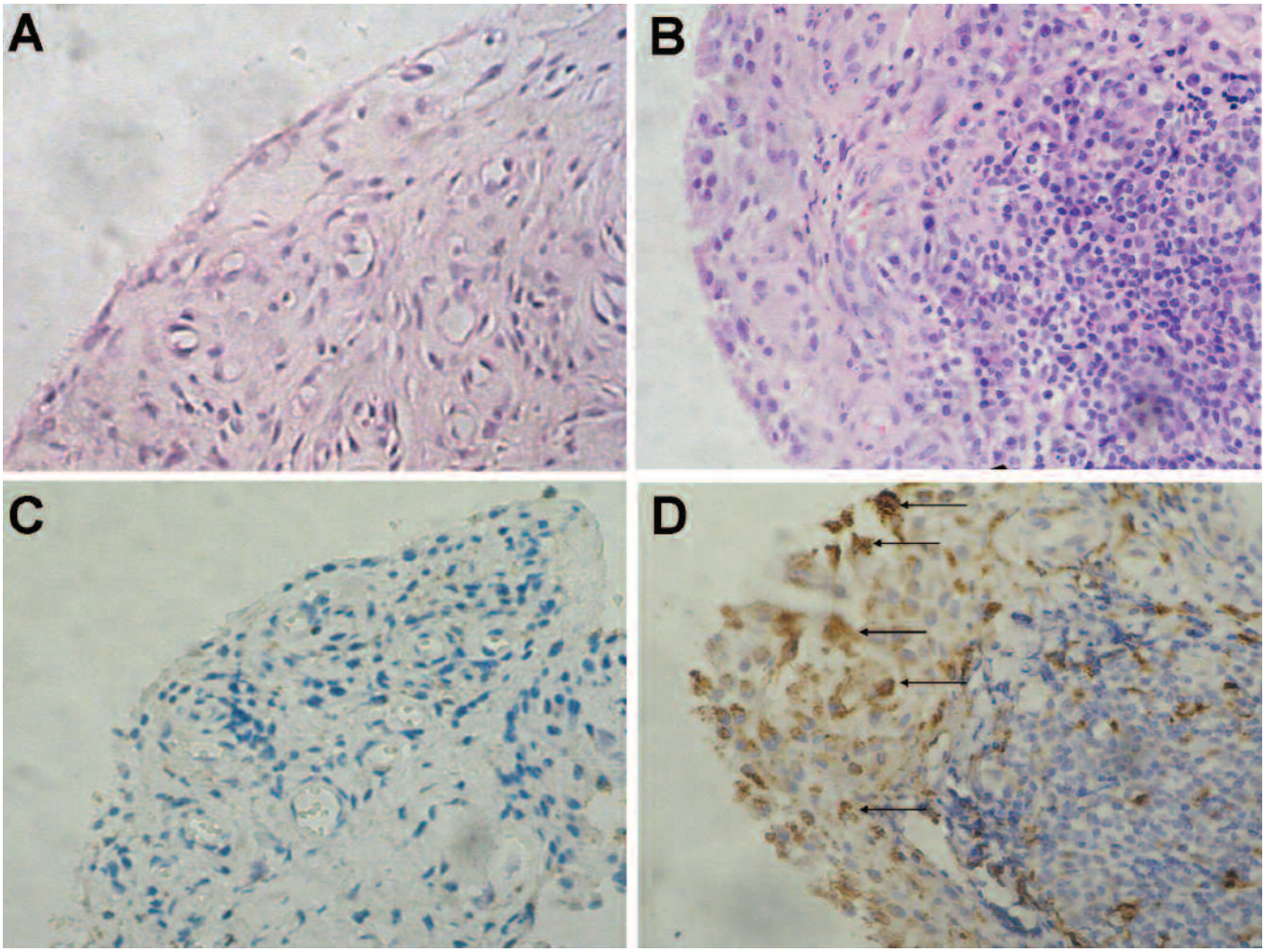

Figure 2. Representative histological and immunohistochemical findings. A. Mild synovitis in an RA patient with low serum GPI level (synovitis score 0.5, serum GPI level 0.01 μg/ml). B. Severe synovitis in an RA patient with high serum GPI level (synovitis score 6.0, serum GPI level 4.02 μg/ml). C. Few CD68+ cells in the lining layer of the synovium from an RA patient with low serum GPI level. D. Higher intensity of CD68+ cells in the lining layer of the synovium from an RA patient with higher serum GPI level. A and B, hematoxylin-eosin stain, original magnification ×400; C and D, anti-CD68 immunostaining, original magnification ×400. Arrows show CD68+ cells located at the lining layer of the synovium.

controls, 0.03 ± 0.05 μg/ml ($p < 0.0001$). As depicted in Figure 3A, the median serum GPI level in the RA group (0.92 μg/ml, range 0.01–11.32 μg/ml) was significantly higher than that in the non-RA disease group (0.01 μg/ml, range 0.01–3.50 μg/ml; $p < 0.0001$). Serum GPI levels were not significantly different between healthy controls and patients with non-RA disease: healthy controls, 0.03 ± 0.05 μg/ml; non-RA patients, 0.19 ± 0.57 μg/ml ($p = 0.561$). Serum soluble GPI concentrations > 0.2 μg/ml were considered to be GPI-positive. The rate of serum GPI positivity was significantly higher in RA patients than healthy controls and patients with non-RA disease: 64.66% (75/116) compared to 1.98% (2/101) and 10.14% (7/69), respectively ($p < 0.0001$).

The 116 patients with RA were divided into 4 groups according to the DAS28-CRP score. The rates of serum GPI positivity in the high, moderate, and low activity RA groups and the RA remission group were 72.9% (35/48), 66.0% (33/50), 44.4% (4/9), and 33.3% (3/9), respectively. Although the rate of serum GPI positivity seemed to decrease when disease activity decreased, there were no significant differences in GPI-positive rates among these 4 groups (Table 3), and there were no significant differences in GPI levels among the 4 groups.

Serum GPI levels in other rheumatic diseases. Serum GPI levels from 69 patients with other non-RA rheumatic diseases were also tested. Seven samples were positive for GPI: 1 of the 15 gout sera (6.7%), 2 of 15 AS sera (13.3%), 1 of 7 OA sera (14.3%), 1 of 3 pSS sera (33.3%), and 1 of 3 polymyalgia rheumatica sera (33.3%), and the one vasculitis serum sample (100%) had GPI levels above 0.2 μg/ml. None of the samples from patients with SLE, unclassified connective tissue disease, undifferentiated spondyloarthritis, dermatomyositis, systemic sclerosis, reactive arthritis, Wegener's granulomatosis, or fasciitis was GPI-positive.

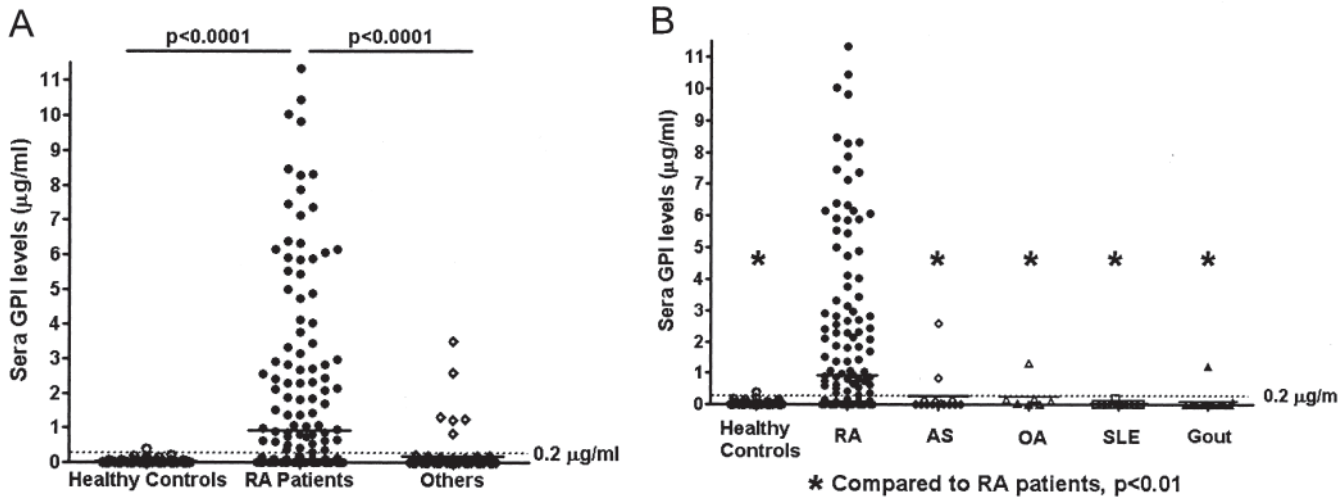

Figure 3. Elevated serum levels of GPI from patients with RA. A. Scatterplots show sera soluble GPI antigenic concentrations in healthy controls (n = 101), RA patients (n = 116), and non-RA rheumatic disease patients (n = 69), measured by a capture ELISA. Each symbol represents the determination for an individual. B. When individual diseases were analyzed separately, significantly higher soluble GPI was detected in RA patients compared to patients with AS, OA, SLE, and gout. Each symbol represents the determination for an individual. Healthy controls, n = 101; RA patients, n = 116; AS patients, n = 15; OA patients, n = 7; SLE patients, n = 11; and gout patients, n = 15. Horizontal lines show the median for each population. Soluble GPI concentrations < 0.2 μg/ml were considered GPI-negative. The p values were calculated using the Kruskal-Wallis one-way analysis.

Table 3. Sera GPI levels in patients with RA who have different disease activity.

DAS28-CRP	Disease Activity	n	GPI-positive Patients, n (%)
> 5.1	High	48	35 (72.9)
3.2–5.1	Moderate	50	33 (66.0)
2.6–3.2	Low	9	4 (44.4)
< 2.6	Remission	9	3 (33.3)

RA: rheumatoid arthritis; GPI: glucose-6-phosphate isomerase; DAS28: Disease Activity Score in 28 joints; CRP: C-reactive protein.

When individual diseases were analyzed separately, significantly higher soluble GPI was detected in RA patients compared to patients with AS, OA, SLE, and gout: RA patients, 2.25 ± 2.82 μg/ml compared to AS patients, 0.26 ± 0.68; OA patients, 0.25 ± 0.47; SLE patients, 0.02 ± 0.05; and gout patients, 0.10 ± 0.31 μg/ml (all p < 0.0001; Figure 3B). No significant difference was found among healthy controls, AS patients, OA patients, SLE patients, and gout patients.

Sensitivity, specificity, and ROC curve for serum GPI in predicting RA. Seventy-five of 116 RA patients (64.7%) were positive for GPI at high concentration, whereas only 9 of 170 control sera (5.3%) showed a positive reaction (p < 0.0001). Sensitivity of serum GPI in diagnosis of RA was 64.7%, and specificity was 94.7% at the cutoff value of 0.2 μg/ml. The receiver operating characteristic (ROC) curve indicated that the serum level of GPI provided an accurate test for defining the disease in RA patients as the area under the curve (AUC) for GPI was 0.837 (p < 0.0001; Figure 4).

Correlations between biomarkers and clinical measures. Spearman rank order correlation test was performed to investigate the correlation between serum GPI levels and ESR, CRP levels, RF levels, anti-CCP antibody levels, TJC in 28 joints, SJC in 28 joints, pain VAS, HAQ, AIMS, morning stiffness, gripping power, and DAS28-CRP, all serological and clinical measures that reflect the activity and severity of RA. In the RA group, only RF and anti-CCP antibodies correlated significantly with serum GPI levels (r = 0.385, r = 0.323, respectively; p < 0.001, p = 0.001). No significant correlation was found between serum GPI level and DAS28-CRP (p = 0.098). In contrast, DAS28-CRP correlated significantly with ESR (r = 0.496, p < 0.0001), CRP (r = 0.399, p < 0.0001), RF (r = 0.233, p = 0.013), anti-CCP antibodies (r = 0.270, p = 0.008), TJC in 28 joints (r = 0.913, p < 0.0001), SJC in 28 joints (r = 0.709, p < 0.0001), pain VAS (r = 0.321, p = 0.001), HAQ (r = 0.441, p < 0.0001), AIMS (r = 0.265, p = 0.017), and gripping power (r = –0.200, p = 0.043). We found no correlation between the presence/absence of serum GPI and age or sex.

In the non-RA rheumatic diseases group, Spearman rank order correlation analysis showed no significant correlation between serum GPI levels and ESR, CRP levels, RF levels, or anti-CCP antibody levels.

Comparative analysis of serum GPI level and RA disease activity in RA patients during followup. Thirty-eight RA patients were followed for 1 to 16 months and underwent reevaluation of clinical status and serum GPI levels. All patients were between 17 and 78 years of age (mean 48.8 ± 14.3 yrs, median 51). Twenty-six of these RA patients were female; 12 were male. Thirteen (34.2%) of the patients were taking prednisone, 37 (97.4%) were taking DMARD:

methotrexate n = 30 (78.9%) and leflunomide n = 27 (71.1%). Eleven (28.9%) patients were taking etanercept, and 2 (5.3%) were taking infliximab. Disease activity and pain decreased at the second visit compared to baseline. After initiation of antirheumatic treatments, GPI levels decreased significantly (2.81 ± 3.12 vs 1.44 ± 2.09 μg/ml; p = 0.016). The paired Wilcoxon signed-rank test showed that serum GPI levels, CRP, ESR, RF, anti-CCP antibody, TJC in 28 joints, SJC in 28 joints, DAS28-CRP, pain VAS, HAQ, and morning stiffness all decreased significantly during followup, with significantly increased gripping power (Table 4).

DISCUSSION

Using quantification of RA synovitis with the synovitis score, as well as ELISA detection of serum GPI levels, we found a significant correlation between elevated serum GPI level and the synovitis score in RA. We also found that RA patients had significantly higher serum GPI levels than healthy controls and non-RA rheumatic disease controls. These results suggested that elevated serum GPI may be involved in the pathogenesis of synovitis of RA.

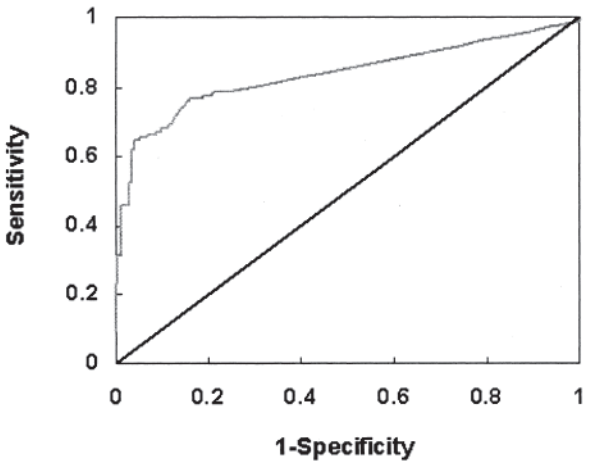

Figure 4. Receiver operating characteristic (ROC) curve illustrating the relationship between the sensitivity and the complement of the specificity (1 – specificity) for the prediction of rheumatoid arthritis. A cutoff value of 0.2 μg/ml for serum GPI has a sensitivity of 64.7%, while maintaining a high specificity (94.7%). Area under the ROC curve of GPI is 0.837 (95% CI 0.784, 0.890, p < 0.0001).

GPI, also known as a phosphoglucose isomerase or phosphohexose isomerase, is a polyfunctional molecule[7,10,38]. Molecular cloning and sequencing has shown that it is identical to AMF[7], neuroleukin[39], maturation factor[10], and myofibril-bound serine proteinase inhibitor[40]. The multiple extracellular functions of GPI include promotion of spinal and sensory neuron survival *in vitro* and stimulation of immunoglobulin production by B cells[38,39]. GPI is also a tumor cell product that promotes cell migration[7] and is capable of mediating differentiation of human myeloid leukemia cells to terminal monocytic cells[10]. Moreover, GPI is a major surface antigen in sperm agglutination[41]. Recent research showed that GPI is a new candidate autoantigen in the initiation of autoimmune arthritis[11,42,43]. The arthritogenicity of GPI was first described in T cell receptor-transgenic K/BxN mice, which showed that GPI could serve as an autoantigen for both B and T cells, as well as an important factor in maintaining disease[11].

GPI, which is secreted by lectin-stimulated T cells, induced Ig synthesis in cultured human peripheral blood mononuclear cells[38], stimulated cell migration[44], and stimulated the differentiation of hemopoietic cells[44]. These functions might enhance the immune response to GPI. GPI could easily bind to the negatively charged structures of the joint[45,46,47], resulting in a locally increased concentration of GPI that might exceed a critical threshold necessary to set off a sustained immune response. The K/BxN model showed that the B cell response against the ubiquitously

Table 4. Comparative analysis of disease activity, pain, and sera GPI levels in 38 patients with RA during followup. P values < 0.05 were considered statistically significant.

Characteristic	Baseline	Followup	p
CRP, mean (range), mg/l	40.0 (3.1–167.0)	18.4 (0.2–118.0)	0.003
ESR, mean (range), mm/h	67.4 (8.0–140.0)	33.7 (7.0–115.0)	< 0.0001
RF, mean (range), U/ml	344.9 (9.1–1580.0)	250.5 (9.1–1570.0)	0.008
Anti-CCP, mean (range), U/ml	141.5 (2.6–300.0)	95.2 (2.8–300.0)	0.034
Swollen joint count, mean (range) of 28 joints	6.1 (0–22)	2.1 (0–22)	< 0.0001
Tender joint count, mean (range) of 28 joints	8.3 (0–28)	2.4 (0–24)	< 0.0001
DAS28-CRP, mean (range)	4.67 (2.43–7.50)	3.11 (1.65–6.19)	< 0.0001
Pain VAS, mm	54 (20–100)	39 (10–100)	< 0.0001
HAQ score	1.06 (0.15–2.55)	0.77 (0–1.65)	0.003
Early morning stiffness, mean (range), min	74.0 (0–300.0)	26.4 (0–180.0)	< 0.0001
Gripping power, mean (range), mm Hg	76.6 (50.0–115.0)	84.8 (50.0–115.0)	0.002
Serum GPI, mean (range), μg/ml	2.81 (0.01–9.81)	1.44 (0.01–9.54)	0.016

RA: rheumatoid arthritis; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; RF: rheumatoid factor; Anti-CCP: anticyclic citrullinated peptide antibodies; DAS28: Disease Activity Score in 28 joints; HAQ: Health Assessment Questionnaire; GPI: glucose-6-phosphate isomerase; VAS: visual analog scale.

expressed GPI was initiated in and focused on the lymph nodes draining the distal joints[48]. Other joint-specific factors such as tumor necrosis factor-α and other cytokines and effector molecules might contribute to the pathogenesis of GPI-induced arthritis[49]. In this study, we showed that serum GPI level correlated positively with the synovitis score and inflammatory cell infiltration of chronic synovitis, as well as the CD68+ cell intensity in the lining layer of RA synovium. These results confirmed the conception that the ubiquitously expressed GPI is involved in the articular inflammatory destruction of RA patients, and that elevated serum GPI levels can trigger the production of proinflammatory cytokines and perpetuate the inflammatory process. As a high-grade synovitis was strongly associated with rheumatic joint diseases[28], and the synovitis score correlated strongly with synovial expression of CD68 (a well validated tissue marker of disease activity in RA[50]), our study also showed that serum GPI level was correlated positively with CD68+ cell intensity in the lining layer of RA synovium, which suggests that higher serum GPI level may participate in the inflammation and joint damage of RA. These results also imply that GPI may play a role in perpetuating arthritis and that blocking GPI response may improve disease outcome. Further studies are needed to evaluate the exact role of GPI in the pathogenesis of RA.

Schubert, *et al* reported that immunization with heterologous GPI in adjuvant induced symmetric polyarthritis of the small distal joints in genetically susceptible normal mice[12], which suggested that systemic autoimmunity induced by ubiquitously expressed Ags could be highly specific for certain organ-specific autoimmune diseases in genetically unaltered mice. To assess whether the level of serum GPI was altered in RA, we investigated soluble GPI levels in sera from a large proportion of patients with RA, and also in sera from patients with other forms of arthritis. Serum soluble GPI levels were significantly higher in RA patients compared with non-RA rheumatic disease patients and healthy controls. These results were consistent with the studies of Schaller, *et al*[13]. In arthritic K/BxN mice, concentrations of free GPI in serum were lower than those in normal mouse serum, but C1q binding complexes (circulating GPI as immune complexes) were at titers comparable to those found in (NZB×NZW)F1 mice, which were used as positive controls, and these C1q binding complexes were only detected after 30 days of age, coincident with onset of arthritis[51]. Our study also detected elevated levels of soluble GPI, in the form of GPI-antigen, or GPI-anti-GPI complexes, in the serum of RA patients, and the increase was specific for RA. We showed that the sensitivity of serum GPI in diagnosis of RA was 64.7%, specificity was 94.7%, with an AUC of 0.837, demonstrating that soluble GPI are disease-specific for RA. Although its low sensitivity does not allow its use as a screening test, due to its high specificity, it may become one of the useful serologic tests for differentiating RA from other diagnoses.

Although we found no significant correlation between serum GPI level and DAS28-CRP, we speculated that it was due to the small number of patients in the low disease activity group and in the remission group, as the p value of the correlation analysis for serum GPI level and DAS28-CRP was 0.098. More patients are needed, especially RA patients with low disease activity or with disease remission, to test a correlation between serum GPI level and disease activity more reliably. In this study, we did find a positive correlation between serum GPI level and RF level as well as anti-CCP antibody level in the RA group, but not in the non-RA rheumatic disease group, which further confirmed the pathogenic role and the specificity of GPI in RA. To pursue the value of serum GPI in assessing disease activity, we did a followup study on serum GPI level and clinical endpoints in 38 RA patients after initiation of treatment. In this followup study, serum GPI levels decreased in parallel with improvement of the clinical and laboratory endpoints we assessed, suggesting that the serum GPI level might reflect disease activity in RA after initiation of treatment and that it could be used to monitor effects of therapeutic interventions.

Our results indicate that increased levels of GPI are frequently found in the serum of patients with RA, and that elevated serum GPI correlates with the histological severity of synovitis in RA. Moreover, serum GPI levels decreased after initiation of therapeutic intervention. Thus, the GPI level might prove useful as a serum biomarker in randomized control trials for assessing the efficacy of novel treatments, and in clinical practice for following disease progression. Future studies are needed to examine whether neutralization of GPI could alleviate the clinical and/or pathological manifestations of RA.

ACKNOWLEDGMENT

We thank all the patients, healthy volunteers, and members of the medical staff who generously collaborated with this research.

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